The PTP Family Photo Album

Michel L. Tremblay[1,*]
[1]Goodman Cancer Centre, Biochemistry Department, Montreal, Quebec H3G 0B1, Canada
*Correspondence: michel.tremblay@mcgill.ca
DOI 10.1016/j.cell.2009.01.006

Protein tyrosine phosphatases (PTPs) are central players in many biological processes. In this issue, Barr et al. (2009) analyze 22 different PTP structures to define their common and unique features. This effort provides key insights into the regulation of PTP activity that could lead to the development of new therapeutics.

Phosphorylated tyrosine residues serve as unique and exquisitely sensitive switches for the initiation and termination of intracellular signals. Although cells have evolved several protein domains that recognize phosphotyrosine in the context of a wide number of proteins, the protein tyrosine phosphatases (PTPs) remain the most divergent and ubiquitously expressed gene families with this capacity. The human genome contains 107 PTPs, which recognize and remove phosphate moieties attached to tyrosine/serine/threonine containing protein substrates. Based on their primary sequence, only 38 of these enzymes appear to be uniquely specific to phosphotyrosine and this group is so divergent that they have been further subclassified into 17 subgroups of enzymes. This diversity likely reflects the richness of the interactions of PTPs with the cell signaling machinery. In this issue, Barr et al. (2009) examine 22 PTP structures and present several new insights into substrate recognition and catalytic activity that will be of interest to both the PTP community and those seeking to identify PTP modulators for the treatment of various diseases.

In the 20 years since their discovery, much of what we have learned about the mechanism of action of the typical PTP catalytic domain comes from crystal structures of a relatively small subset of these proteins. The PTP catalytic domains of these enzymes are generally easily purified and obtained in very active extracts. And although they display poor substrate specificity in vitro, they are extremely specific in vivo. This was recently demonstrated for PTP1B, which recognizes only one of multiple tyrosine phosphorylation sites on cortactin, a protein that regulates actin branching (Stuible et al., 2008). Therefore, to reveal their common features, as well as the uniqueness of the different subgroups of PTPs, an assessment of a larger pool of PTP structures has been needed.

Barr et al. (2009) have now analyzed the variation in surface properties of 22 human PTP family members. In addition to the many individual particularities that are revealed about each, they present the discovery of a new atypical conformation for the catalytic (WPD) loop in which the loop is open even when the catalytic pocket is bound by substrate. They also assay a broad range of phosphopeptide substrates and model some of those peptides onto PTP structures. Two sets of findings particularly stand out from their work. First, the systematic comparison of these different PTPs allowed the authors to show that a secondary substrate-binding pocket, initially reported for PTP1B (Barford et al., 1994), can also be found in most other PTPs. The secondary substrate-binding site is separated from the catalytic pocket by a small gateway region, which consists of a direct groove between the catalytic pocket and the secondary substrate binding site that is flanked by two bulky residues. Barr and colleagues examined these two features in all of their PTP structures and were able to categorize most of their candidate PTPs into five subsets of enzymes based on whether those two structural elements were "open" or "closed." For example, they observed that the secondary substrate-binding site could be anchored on a basic residue (Arg24 in PTP1B) or a cysteine for the R8 subgroup (IA2, IA2β). Alternatively, other PTPs, such as STEP and HePTP, possess an aromatic or a proline residue at that site that eliminates the possibility of the region acting as a substrate-binding pocket. Moreover, they note that the gateway region is either "open" with sufficient space for a peptide to come into the groove or "closed" by the presence of bulky residues that prevent direct access between the secondary substrate-binding pocket and the catalytic pocket. The gateway region and the secondary substrate-binding site are likely to be crucial for substrate specificity. Importantly, PTP1B is already targeted by inhibitors that simultaneously obstruct the catalytic pocket and the secondary substrate-binding pocket (Zhang and Zhang, 2007). The authors' recognition that most members of the PTP family have both a catalytic pocket and a secondary substrate-binding site provides an interesting and favorable rationale for the further development of such inhibitors.

A second general feature of PTPs that is clarified by this new work is how homodimerization of receptor PTPs inhibits their activity. Receptor-like PTPs (RPTPs) comprise an extracellular segment, a transmembrane region, and an intracellular section containing one or two catalytic domains. In contrast to receptor tyrosine kinases that are activated by pairing, dimerization of RPTPs inhibits their activity. A mechanism for their inhibition through dimerization was first proposed by Bilwes et al. (1996) who noticed that RPTPα D1 crystals are indeed dimers. In their structure, a segment of 24 amino acids derived from a region between the transmembrane domain and the N terminus of each D1 domain, named the

"wedge," directly interacts with the D1 catalytic pocket of the partner protein. This results in reciprocal steric hindrance of the catalytic pockets (Figure 1). This model was supported by other work, such as by Majeti et al. (1998), who showed that a single mutation in this wedge domain increases the in vivo activity of a hybrid EGFR-CD45. Xie et al. (2006) also reported that peptides derived from the wedge region of LAR (leukocyte antigen-related protein tyrosine phosphatase) and PTPμ mediate homophilic interactions and reduce homodimerization, resulting in an increase in PTP catalytic activity.

Based on their study, Barr et al. (2009) reject the existence of such an inhibitory wedge domain. In contrast to the "wedge" model, the RPTPγ crystal of the complete intracellular region reveals an interaction between the D1 domain of one of the partners in the dimer with the D2 domain of the other. They recognize the region between the D1 domain and the transmembrane domain not as a wedge but as a linker segment that provides sufficient flexible space to allow turning of the full PTP internal domain by 90°. Turning permits a head-to-toe interaction with the other partner in the dimer. D1-D2 recognition had also been observed by Wallace et al. (1998), who used the D1 domain of RPTPσ as bait in a two-hybrid screen and identified the D2 domain of the close homolog RPTPδ as an interacting partner. This D1-D2 interaction was considerably weakened by the removal of the wedge domain from the D1 bait construct. Yet this finding and the biological effects of wedge-derived peptides observed by Xie et al. (2006) still hint at a possible contribution of this intracellular region to the dimerization process.

The work of Barr et al. allows the examination of a plethora of posttranslational modifications that control PTP activity, including phosphorylation, ubiquitination, proteolytic cleavage, and oxidation. Oxidation of PTPs, for example, causes various conformational changes that have a major impact on enzyme activity. Indeed, Groen et al. (2008) recently confirmed that oxidation of RPTPs causes extensive conformational changes in the cytoplasmic domains that stabilize the inactive state. Further progress in crystallography will eventually lead to examination of the role of the transmembrane domain of receptor PTPs, such as the transmembrane domain of PTPRR that is reported to promote the dimerization process (Noordman et al., 2008). The availability of a growing pool of PTP structures is of great interest, and the 22 new structures concurrently published by Barr and colleagues will have an extraordinary impact on the daily efforts of a great number of researchers working on PTPs. In particular, those seeking to develop new PTP inhibitors stand to gain from this expanded toolbox of structures, bringing the broad clinical application of therapeutics that target PTPs one step closer.

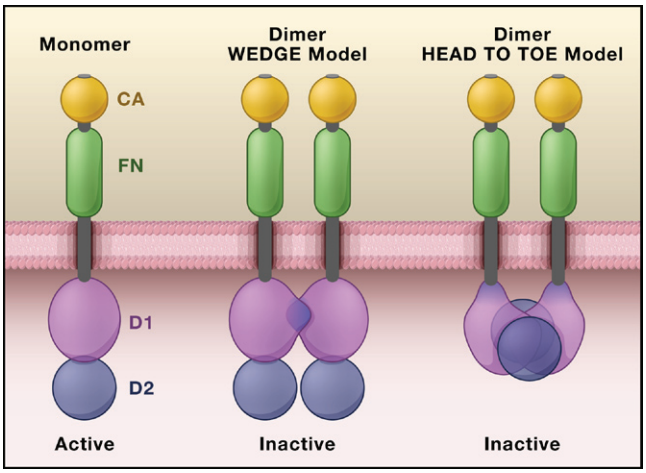

Figure 1. Dimerization Models of a Receptor Protein Tyrosine Phosphatase.

(Left) Depiction of a (R5-like) monomeric active receptor protein tyrosine phosphatase (RPTP) containing an extracellular segment with a carbonic anhydrase motif (CA) and a fibronectin-like element (FN). The extracellular portion is linked through a transmembrane segment to the intracellular domain composed of a proximal catalytic motif (D1) and a distal catalytic motif (D2). (Middle) An inactive PTP dimer is shown based on the "wedge" model of inhibition that results from D1-D1 interactions. (Right) An inactive PTP dimer is shown based on the "head-to-toe" model of inhibition in which the D1 domain of each monomer interacts with the D2 domain of the other monomer.

REFERENCES

Barford, D., Flint, A.J., and Tonks, N.K. (1994). Science *263*, 1397–1404.

Barr, A.J., Ugochukwu, E., Lee, W.H., King, O.N.F., Filippakopoulos, P., Alfano, I., Savitsky, P., and Burgess-Brown, N.A., Müller, S., and Knapp, S. (2009). Cell, this issue.

Bilwes, A.M., den Hertog, J., Hunter, T., and Noel, J.P. (1996). Nature *382*, 555–559.

Groen, A., Overvoorde, J., Van der Wijk, T., and den Hertog, J. (2008). FEBS J. *275*, 2597–2604.

Majeti, R., Bilwes, A.M., Noel, J.P., Hunter, T., and Weiss, A. (1998). Science *279*, 88–91.

Noordman, Y.E., Augustus, E.D., Schepens, J.T., Chirivi, R.G., Ríos, P., Pulido, R., and Hendriks, W.J. (2008). Biochim. Biophys. Acta *1783*, 275–286.

Stuible, M., Dubé, N., and Tremblay, M.L. (2008). J. Biol. Chem. *283*, 15740–15746.

Wallace, M.J., Fladd, C., Batt, J., and Rotin, D. (1998). Mol. Cell Biol. *18*, 2608–2616.

Xie, Y., Massa, S.M., Ensslen-Craig, S.E., Major, D.L., Yang, T., Tisi, M.A., Derevyanny, V.D., Runge, W.O., Mehta, B.P., Moore, L.A., et al. (2006). J. Biol. Chem. *281*, 16482–16492.

Zhang, S., and Zhang, Z.Y. (2007). Drug Discov. Today *12*, 373–381.